## Figure 1

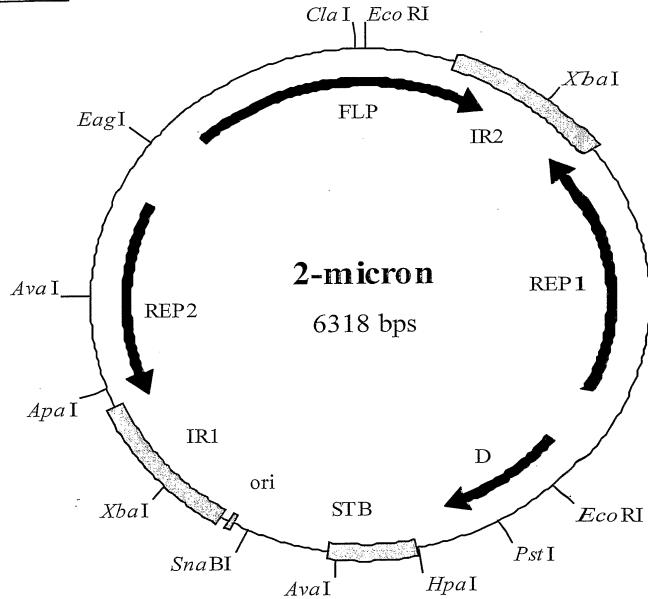
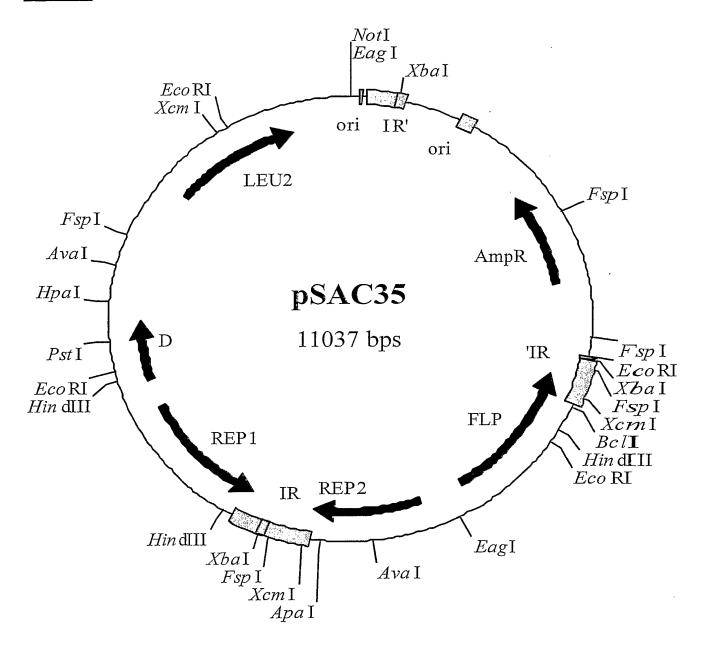
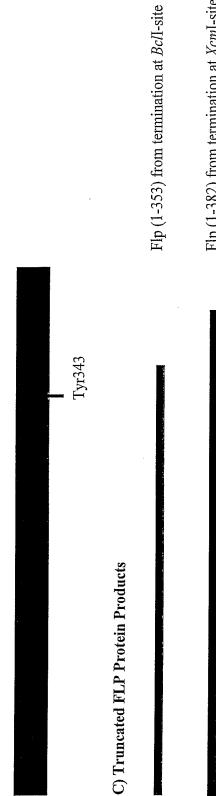


Figure 2



FRT Inverted Repeat (599 bp) FspIA) Restriction Endonuclease Sites used for DNA Insertions in FLP and the FLP Inverted Repeat Xcml Hgal BcIIFigure 3 FLP Coding Sequence (1272 bp)



B) Flp Protein (423 amino acid residues)

Figure 4

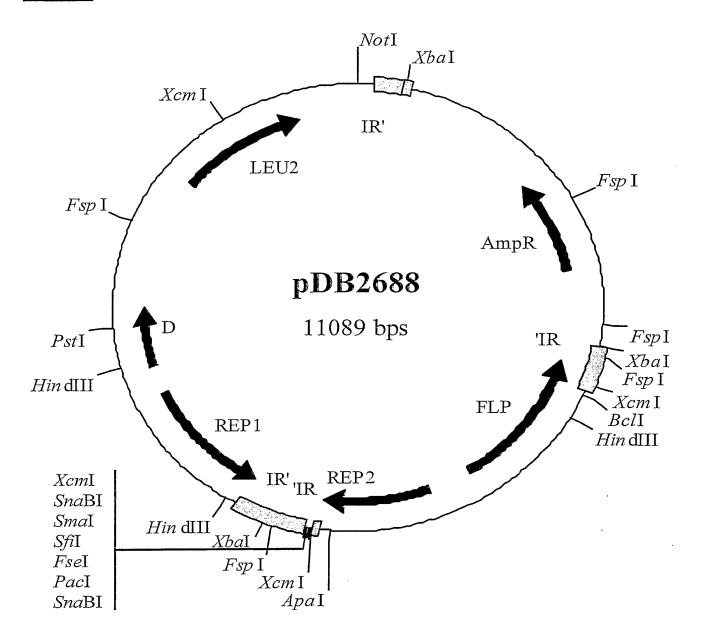


Figure 5

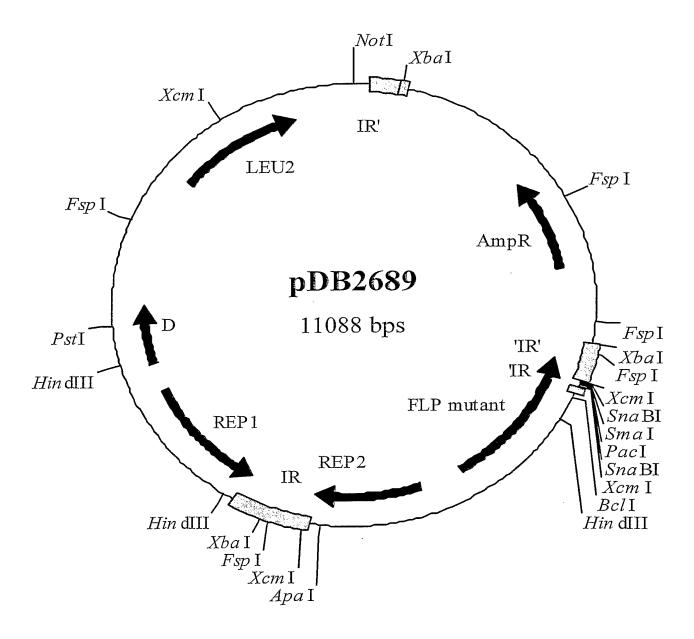


Figure 6

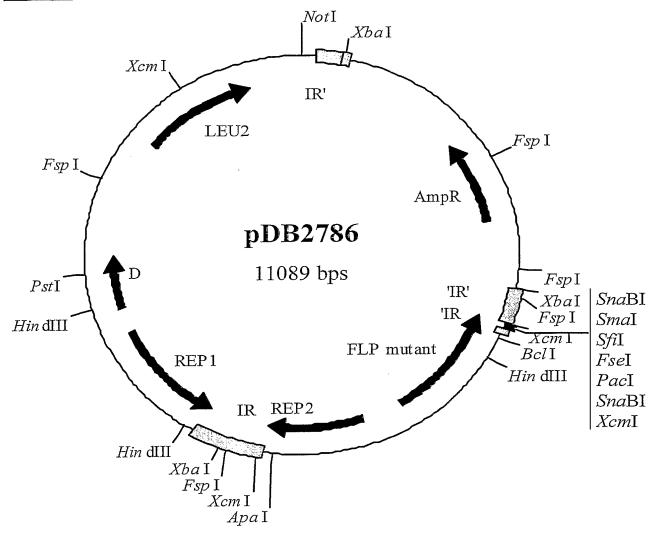


Figure 7

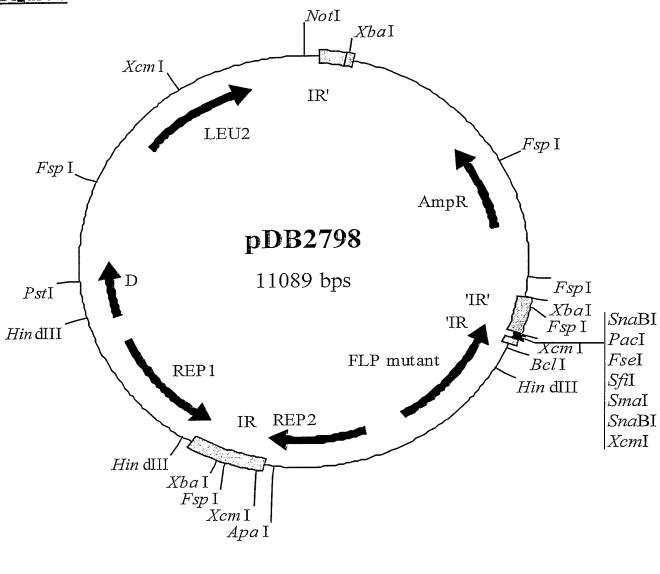
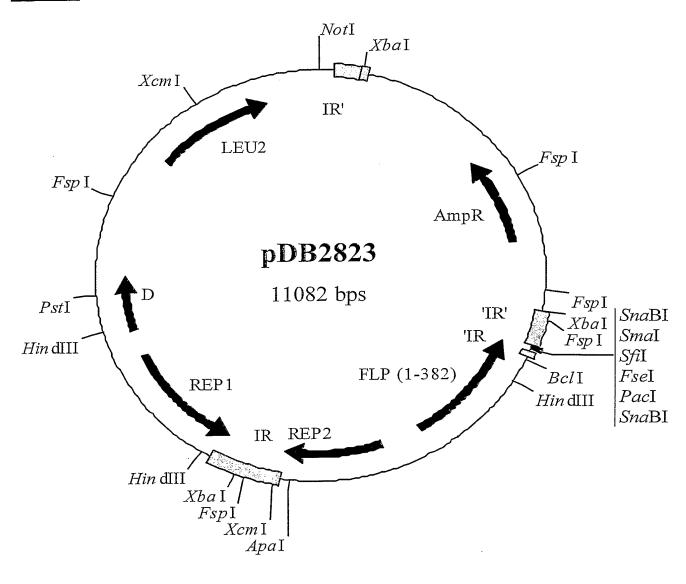


Figure 8



## Figure 9

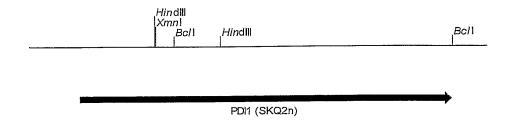


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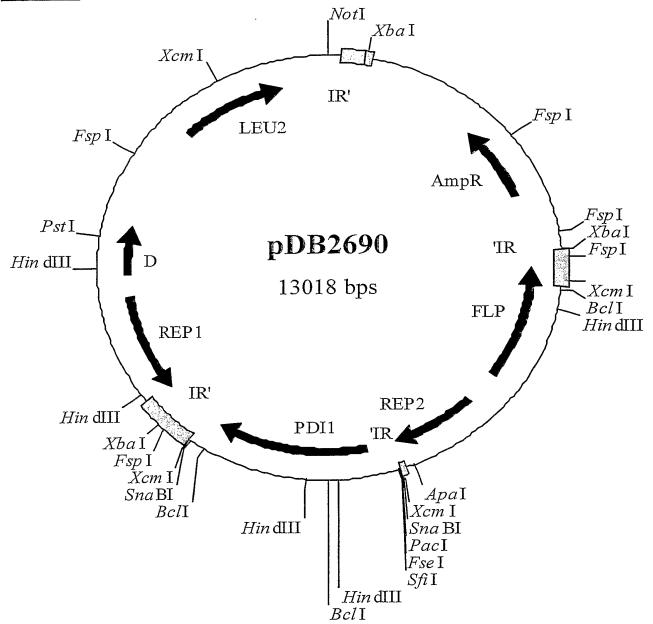
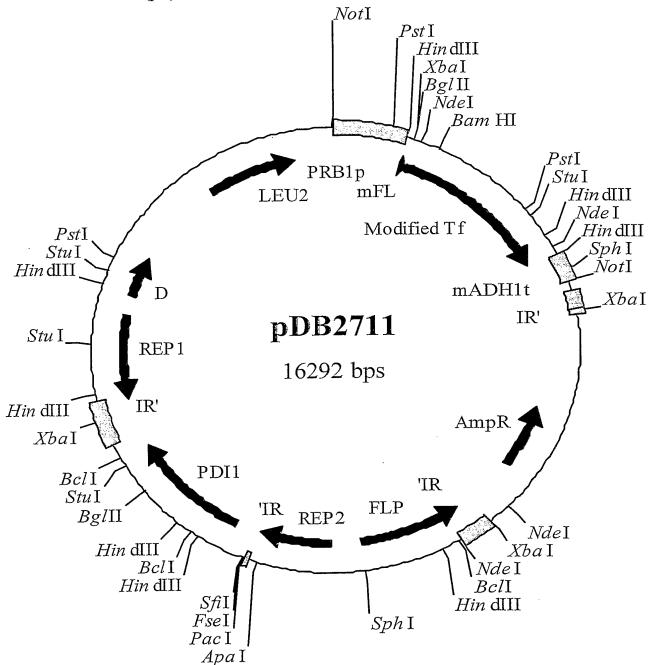


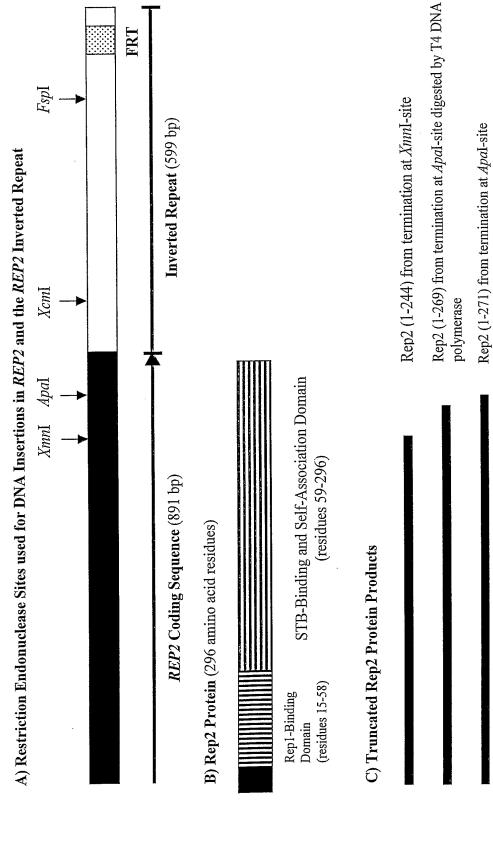
Figure 11

 $mFL = modified HSA(pre)/MF\alpha1(pro)$  fusion leader sequence



5

Figure 12



## Figure 13

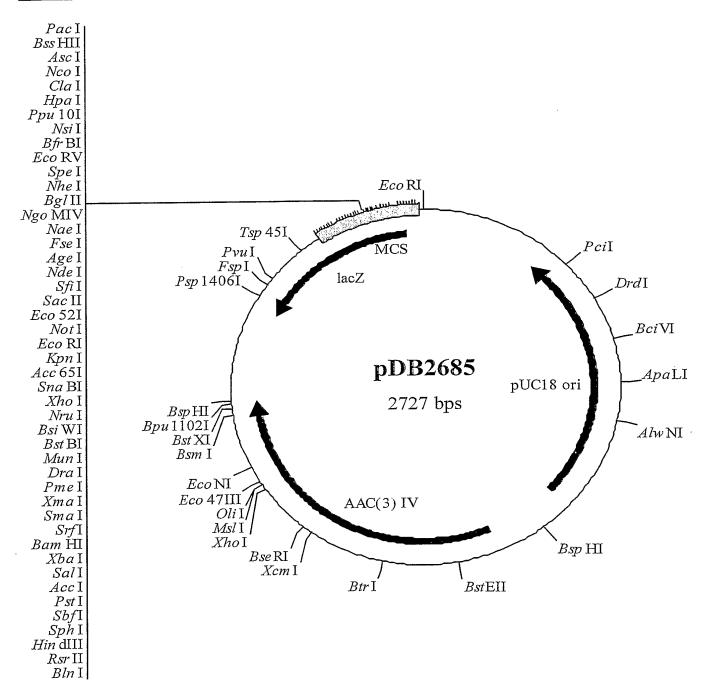


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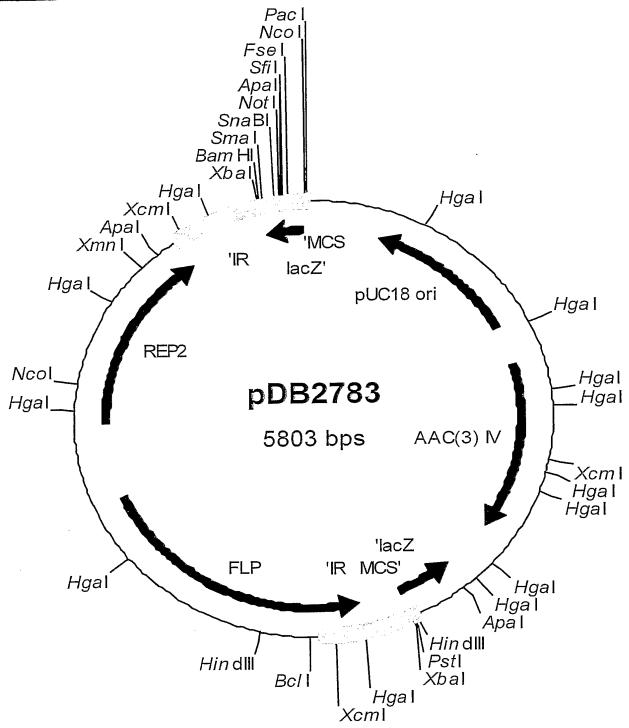


Figure 15

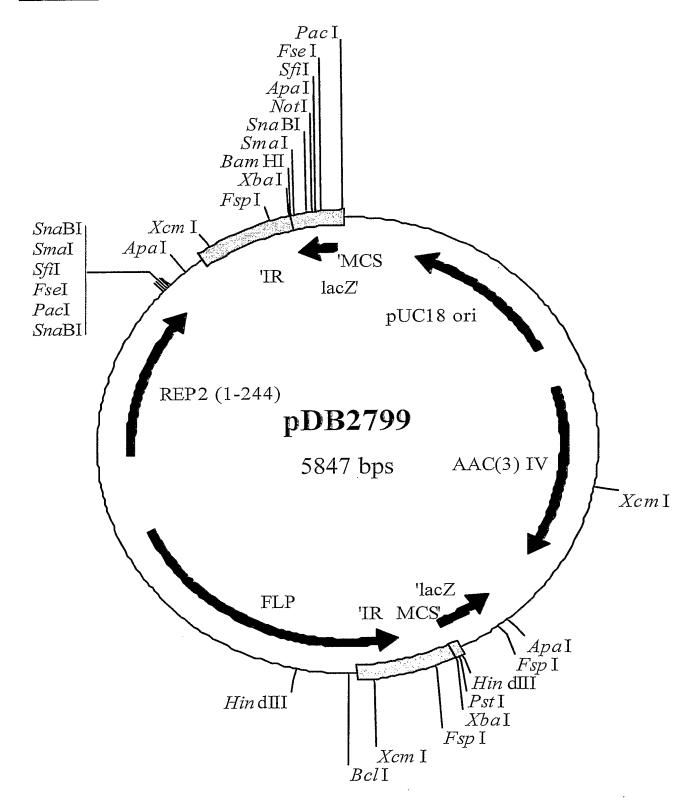


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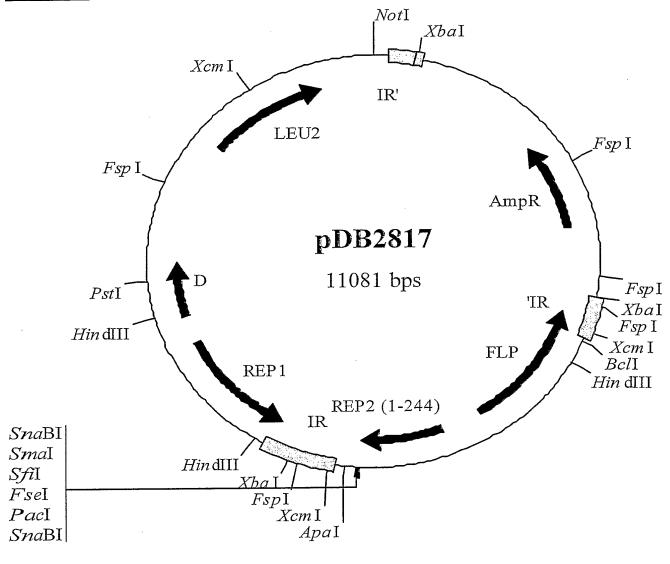


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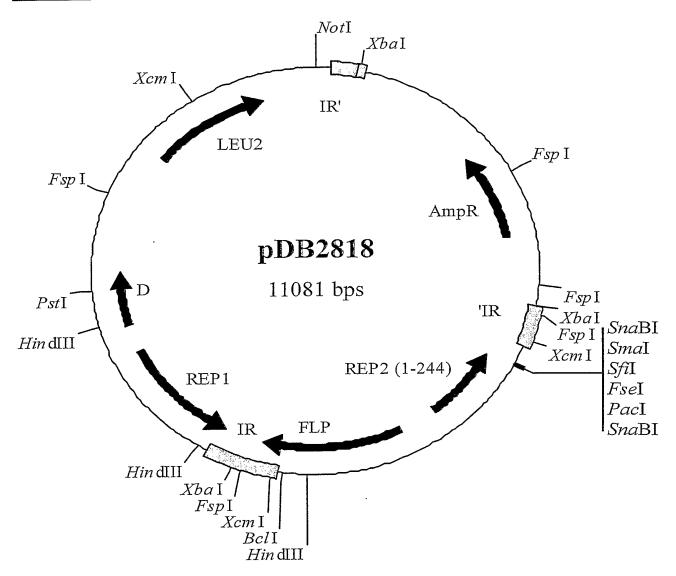
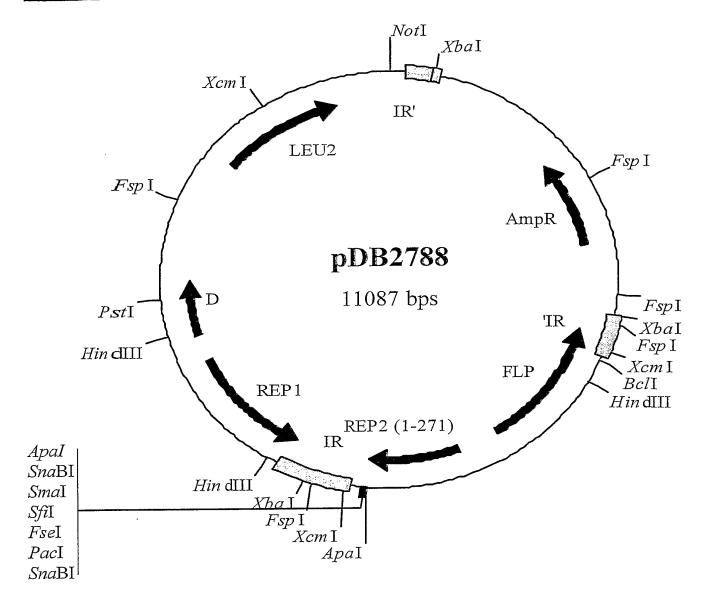


Figure 18



## Figure 19

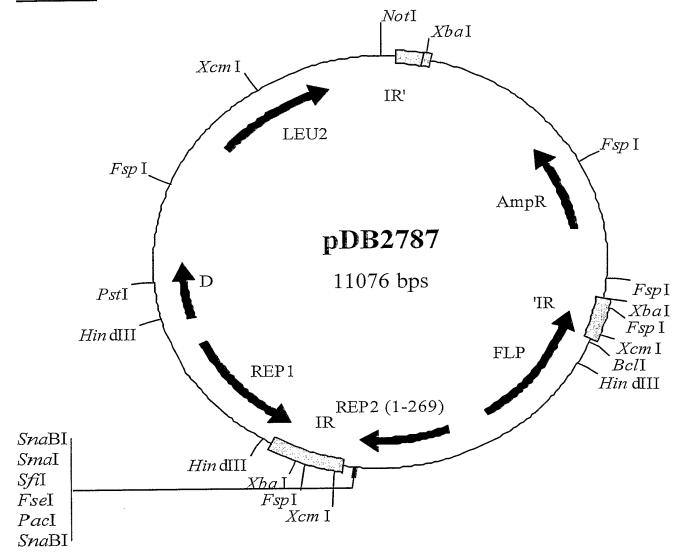


Figure 20

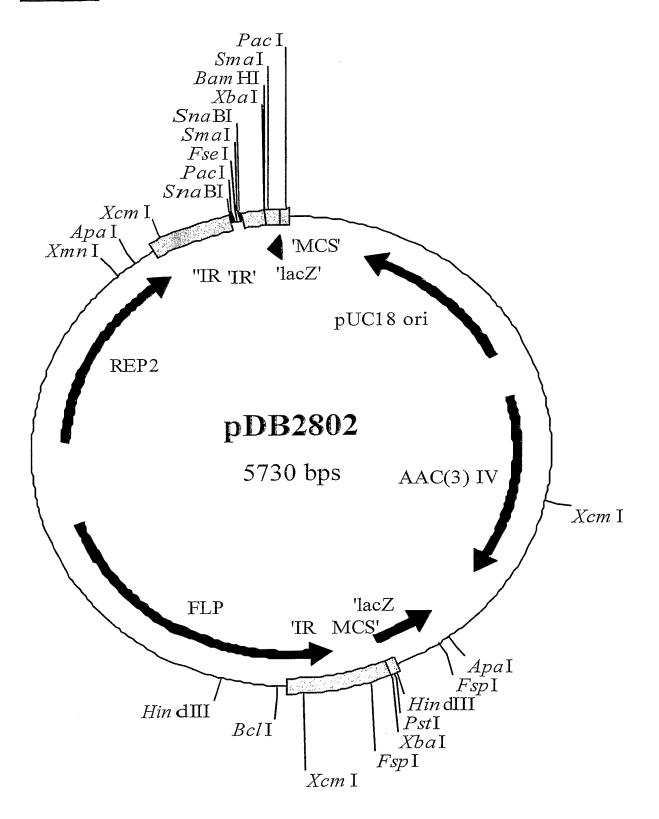


Figure 21

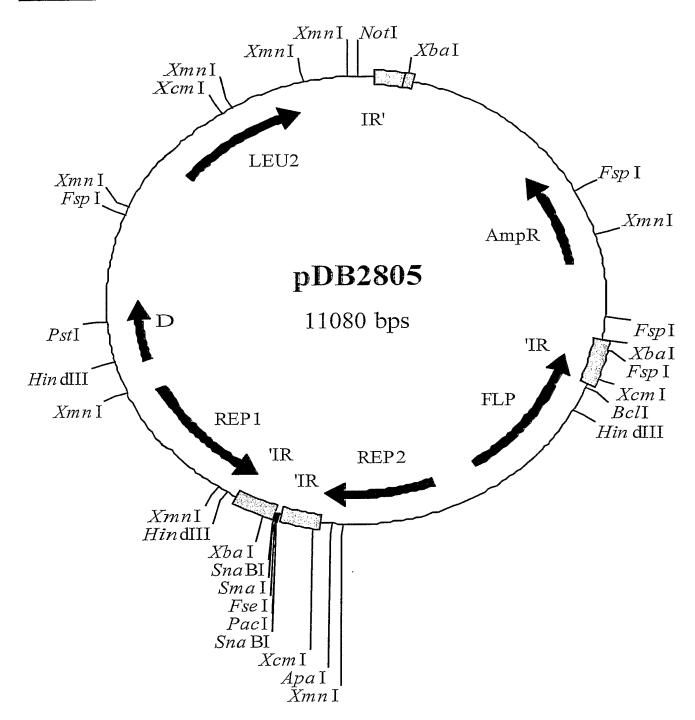


Figure 22

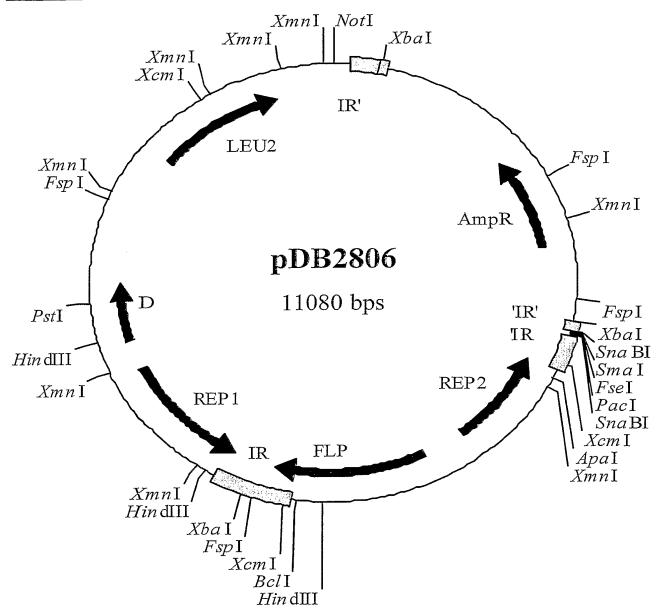


Figure 23

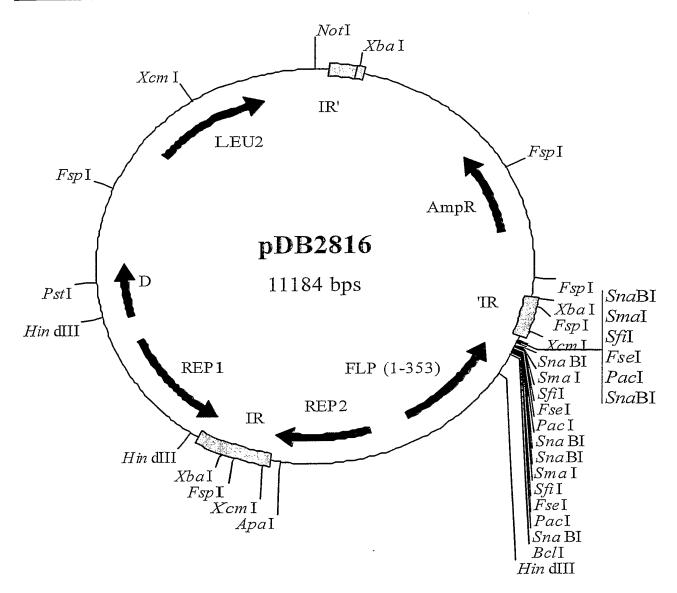


Figure 24

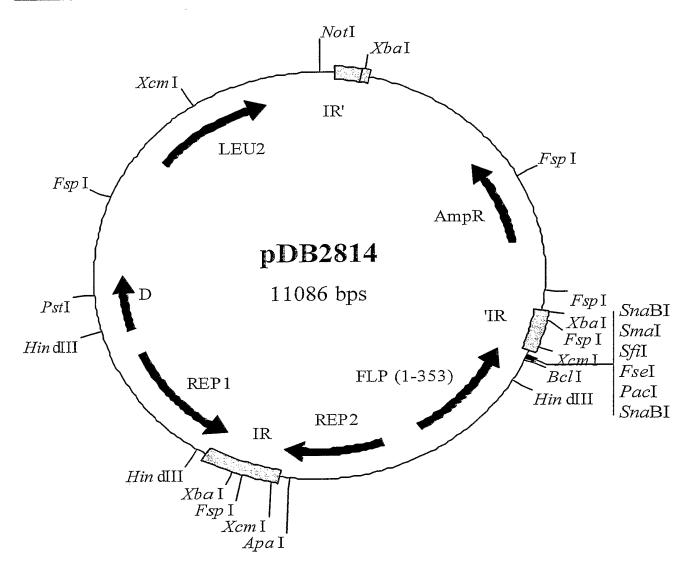


Figure 25

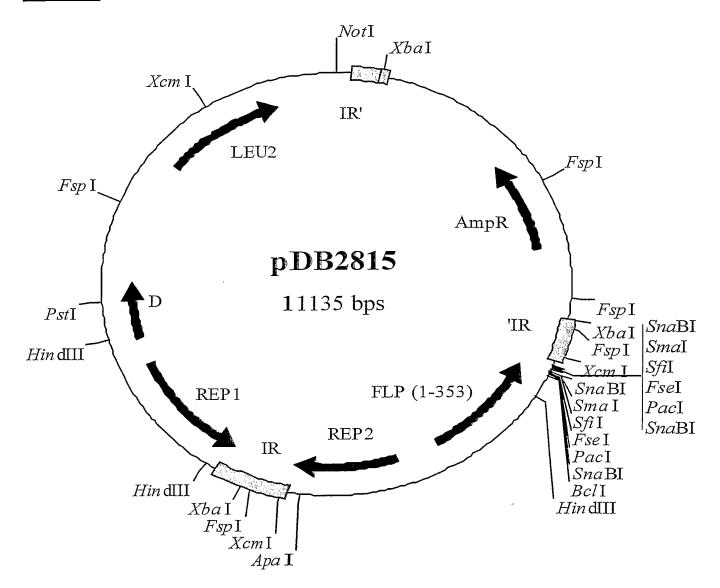


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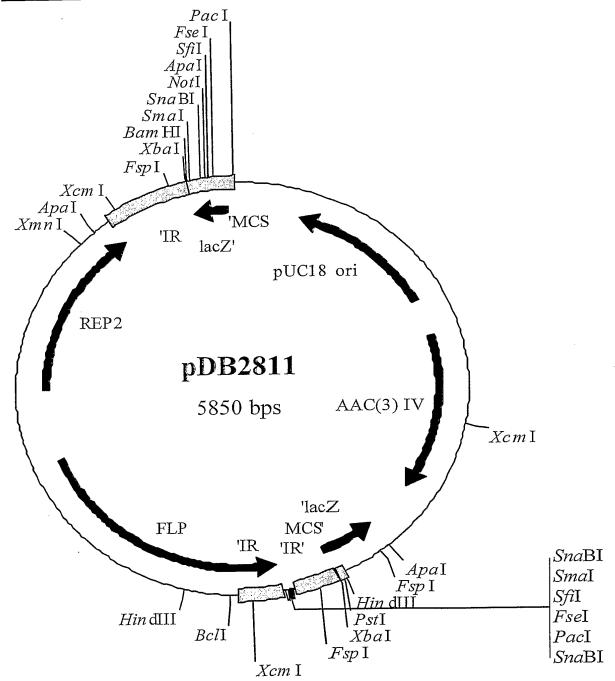


Figure 27

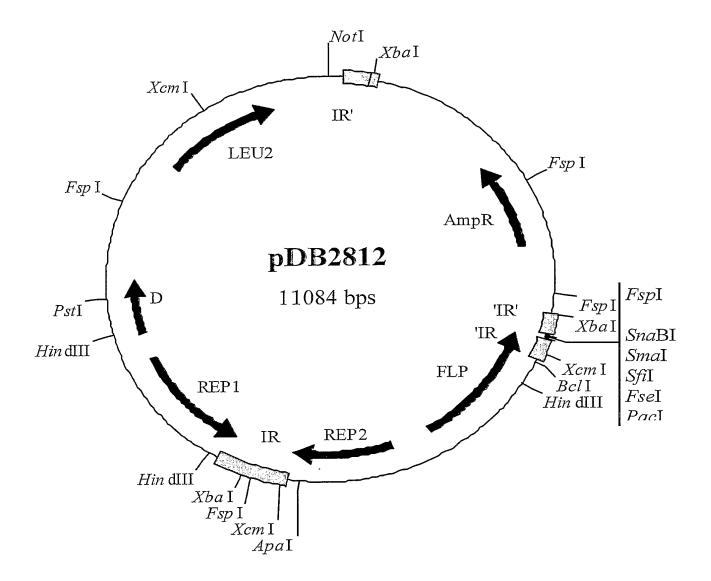


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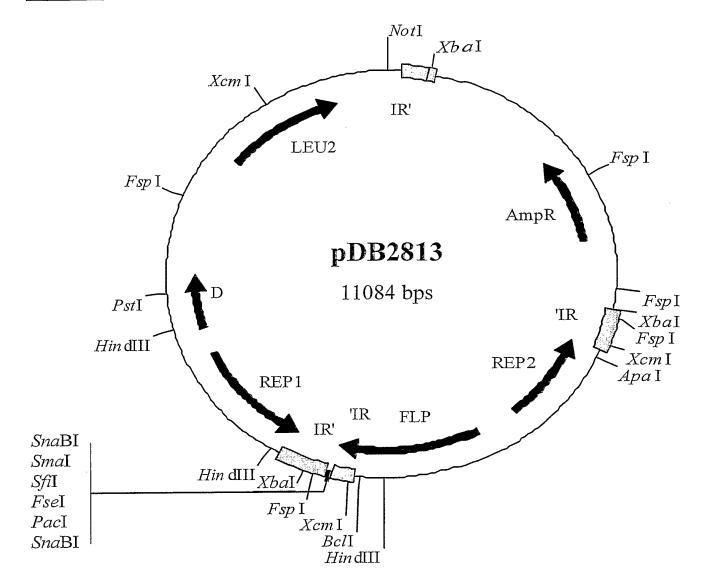


Figure 29

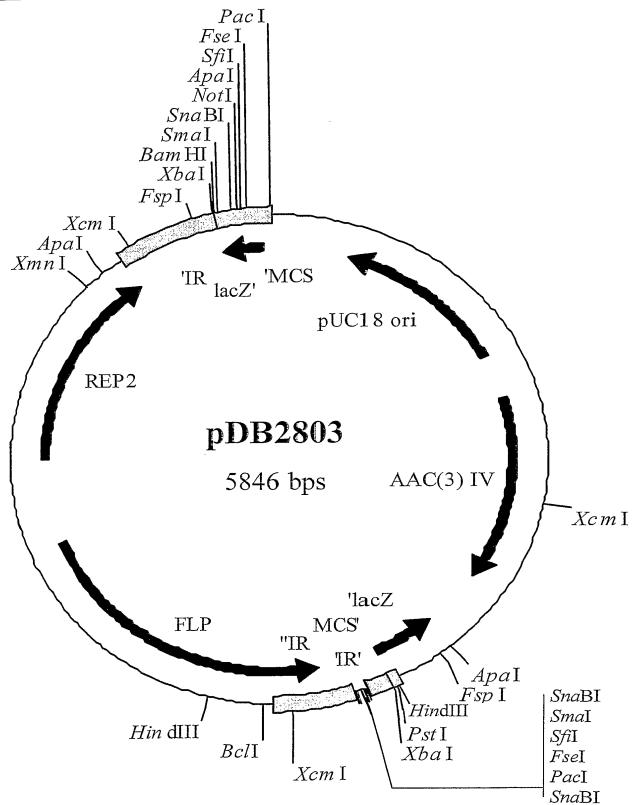


Figure 30

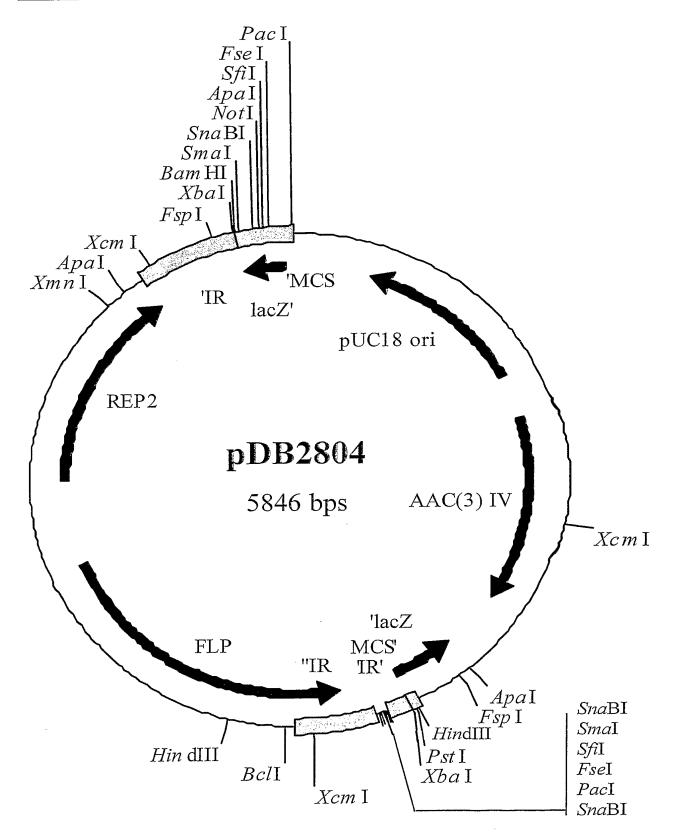


Figure 31

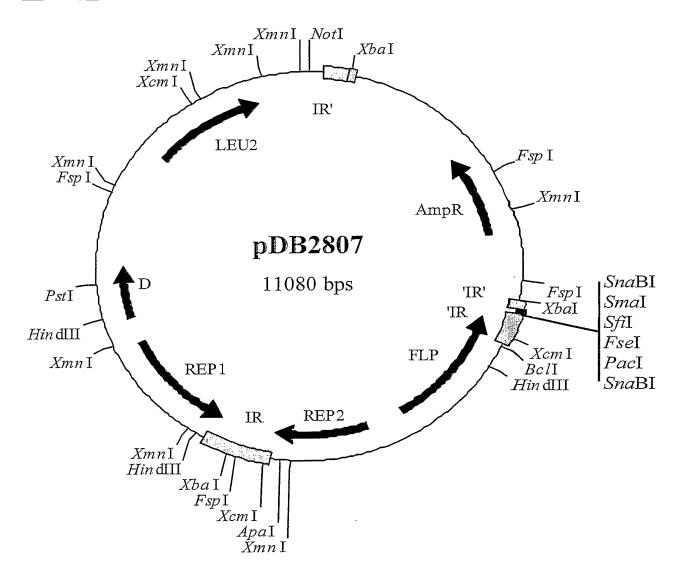
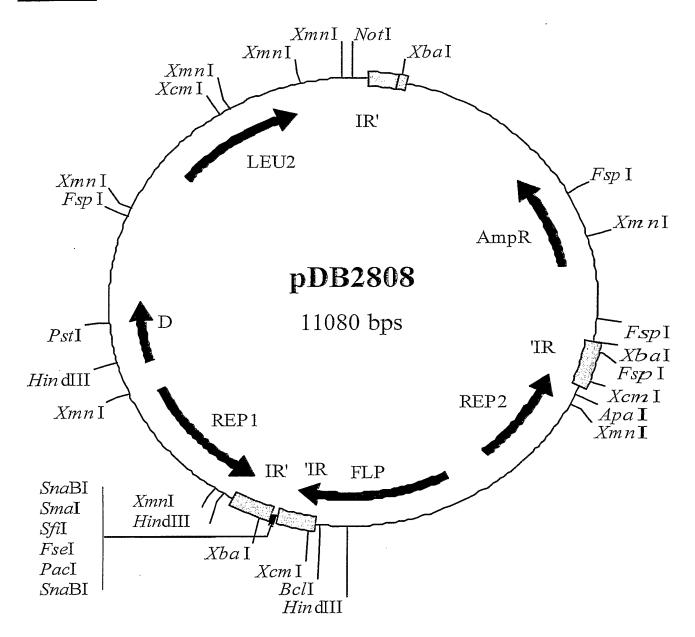


Figure 32



igure 33A

Table 3 (part 1)

Plasmid	Insertion	Insertion Site Details	2µm	Initial	Leu	Leucine Prototrophs	sydo.
	anc		Form	00900	Number	%	Average
pSAC35	1	ı	В	0.074	100/100	100%	
pSAC35	ı	\$	В	0.068	86/56	%16	%66
pDB2818	XmnI	REP2 (1-244)	A	0.062	32/100	32%	
pDB2818	XmnI	REP2 (1-244)	A	0.059	52/100	52%	42%
pDB2787	Apal/T4 pol.	REP2 (1-269)	В	0.054	34/100	34%	
pDB2787	Apal/T4 pol.	REP2 (1-269)	В	0.084	45/100	45%	45%
pDB2788	Apal	REP2 (1-271)	В	990.0	23/100	23%	
pDB2788	Apal	REP2 (1-271)	В	0.051	43/100	43%	33%

Figure 33B

Table 3 (part 2)

Average 100% 100% 100% %19 %19 35% Leucine Prototrophs 100% 100% 100% 100% 100% 100% %69 70% 36% 34% 64% 64% % 100/100 Number 100/100 100/100 100/100 100/100 100/100 36/100 34/100 64/100 69/100 70/100 64/100 Initial OD<sub>600</sub> 0.068 0.055 0.0660.0690.078 0.080 0.057 0.073 0.070 0.0630.082 0.067 2µm Form В  $\mathbf{B}$  $\mathbf{B}$  $\mathbf{B}$ B В  $\mathbf{g}$ ₹  $\mathbf{B}$  $\mathbf{B}$  $\square$ Ą FLP (1-353), 2× Insert FLP (1-353), 1× Insert FLP (1-353), 1× Insert FLP (1-353), 2× Insert Insertion Site Details Inverted Repeat Inverted Repeat Inverted Repeat Inverted Repeat Inverted Repeat Inverted Repeat REP2 (1-244) REP2 (1-244) Insertion Xcml Xcml Xmml Xmnl Site FspIFspIFspIFspIBcIIBcIIBcIIBcIIpDB2805 pDB2805 pDB2814 pDB2815 pDB2815 PDB2688 pDB2806 pDB2806 pDB2814 pDB2817 pDB2817 pDB2688 Plasmid

igure 33C

Table 3 (part 3)

Plasmid	Insertion Site	Insertion Site Details	2µm Form	Initial OD <sub>600</sub>	Leu	Leucine Prototrophs	ophs
					Number	%	Average
pDB2816	BcII	FLP (1-353), 3× Insert	В	0.069	67/100	67%	1
pDB2816	BcII	FLP (1-353), 3× Insert	В	0.056	81/100	81%	74%
pDB2689	XcmI	C-terminal FLP Mutant (FLP 1-384, plus 56 other residues)	В	0.054	73/100	73%	\ c
pDB2689	XcmI	C-terminal FLP Mutant (FLP 1-384, plus 56 other residues)	В	0.056	77/100	77%	/2%
pDB2786	XcmI	C-terminal FLP Mutant (FLP 1-384, plus 14 other residues)	В	0.079	73/100	73%	70177
pDB2786	XcmI	C-terminal FLP Mutant (FLP 1-384, plus 14 other residues)	В	0.052	61/100	61%	0/./0
pDB2823	XcmI	FLP (1-382)	В	0.071	70/100	20%	
pDB2823	Xcml	FLP (1-382)	В	0.055	57/100	57%	64%

igure 33D

<u>Table 3</u> (part 4)

Plasmid	Insertion	Insertion Site Details	711m	Initial	nəT	Leucine Prototrophs	sydo.
	Site		Form	OD <sub>600</sub>	Number	%	Average
pDB2813	Hgal	Inverted Repeat	A	0.057	100/100	100%	0
pDB2813	Hgal	Inverted Repeat	A	0.076	100/100	100%	100%
pDB2808	FspI	Inverted Repeat	A	0.058	100/100	100%	
pDB2808	FspI	Inverted Repeat	A	0.060	100/100	100%	100%
pDB2812	Hgal	Inverted Repeat	B	0.062	100/100	100%	
pDB2812	Hgal	Inverted Repeat	В	0.071	100/100	100%	100%

# Figure 3.

SEQ ID NO:1

$\leftarrow$	AIGGACGACA ITGAAACAGC CAAGAAICIG ACGGIAAAAG CACGIACAGC ITAIAGCGIC IGGGAIGIAI GICGGCIGII IAIIGAAAIG AIIGCICCIG	
101		
201	IGGICTIGAT ICTAGCGCAG AAGATICCAG CGIAICTICI GACICCAGIG CIGAGGIAAI TITGCCIGCI GCGAAGAIGG TTAAGGAAAG GITIGAIICG	
301	ATTGGAAATG GTATGCTCTC TTCACAAGAA GCAAGTCAGG CTGCCATAGA TTTGATGCTA CAGAATAACA AGCTGTTAGA CAATAGAAAG CAACTATACA	1
401	AAICTAITGC TATAATAATA GGAAGAITGC CCGAGAAAGA CAAGAAGAG GCTACCGAAA IGCTCATGAG AAAAATGGAT TGTACACAGT TAITAGTCCC	<i>r</i> \
501	ACCAGCICCA ACGGAAGAAG AIGITAIGAA GCICGIAAGC GICGIIACCC AAIIGCIIAC ITIAGIICCA CCAGAICGIC AAGCIGCIII AAIAGGIGAI	E.,
601	TIATICAICC CGGAAICICI AAAGGAIAIA IICAAIAGII ICAAIGAACI GGCGGCAGAG AAICGITIAC AGCAAAAAA GAGIGAGITG GAAGGAAGGA	~I
701	CTGAAGTGAA CCATGCTAAT ACAAATGAAG AAGTTCCCTC CAGGCGAACA AGAAGTAGAG ACACAAATGC AAGAGGAGCA TATAAATTAC AAAACACCAT	<u></u>
801	CACTGAGGGC CCTAAAGCGG ITCCCACGAA AAAAAGGAGA GIAGCAACGA GGGIAAGGGG CAGAAAATCA CGIAAIACIT CTAGGGIAIG AICCAAIAIC	۲)
901	AAAGGAAAIG AIAGCAITGA AGGAIGAGAC IAAICCAAII GAGGAGIGGC AGCAIAIAGA ACAGCIAAAG GGIAGIGCIG AAGGAAGCAI ACGAIACCCC	ε ).
1001	GCATGGAATG GGATAATATC ACAGGAGGTA CTAGACTACC TTTCATCCTA CATAAATAGA CGCATATAAG TACGCATTTA AGCATAAACA CGCACTATGC	۲١
1101	CGIICIICIC AIGIAIAIAI AIAIACAGGC AACACGCAGA IAIAGGIGCG ACGIGAACAG IGAGCIGIAI GIGCGCAGCI CGCGIIGCAI IIICGGAAAC	۲١
1201	GCICGIIIIC GGAAACGCII IGAAGIICCI AIICCGAAGI ICCIAIICIC IAGAAAGIAI AGGAACIICA GAGCGCIIII GAAAACCAAA AGCGCICIGA	₩.
1301	AGACGCACTI ICAAAAAACC AAAAACGCAC CGGACIGIAA CGAGCIACIA AAAIAIIGCG AAIACCGCII CCACAAACAI IGCICAAAAG IAICITIIIG	ניז
1401	CTATATATCT CIGIGCTATA ICCCTATATA ACCIACCCAT CCACCTTTCG CICCTTGAAC TIGCATCTAA ACTCGACCTC TACAT	

## ligure 3.

SEQ ID NO:2

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Figure 36

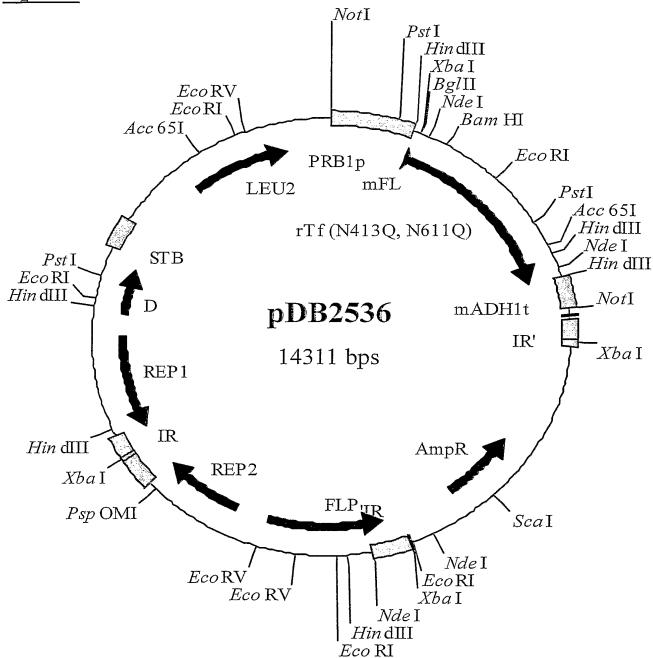


Figure 37

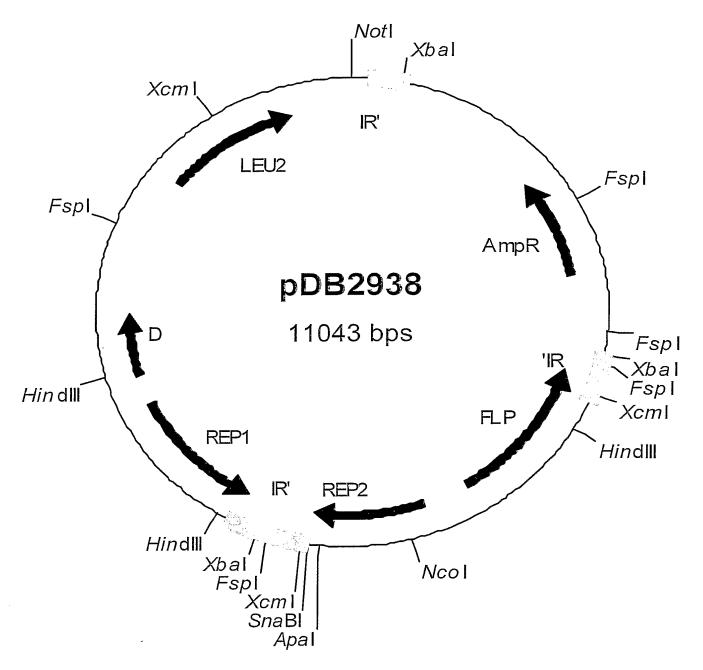


Figure 38

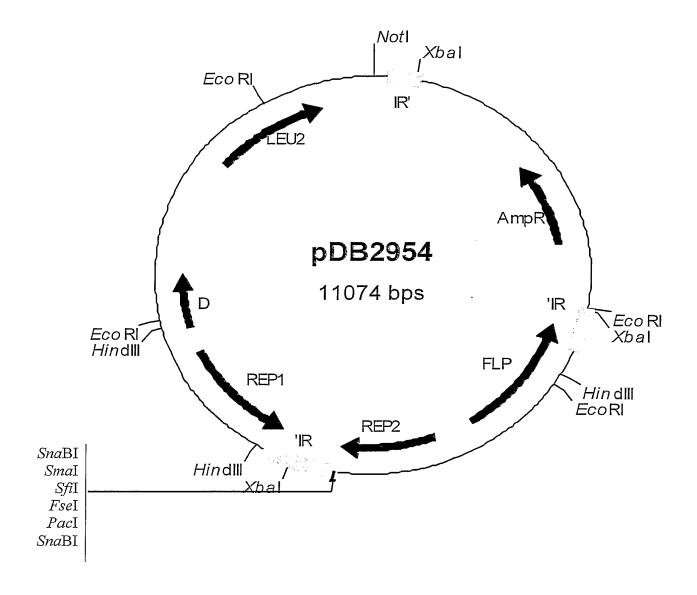


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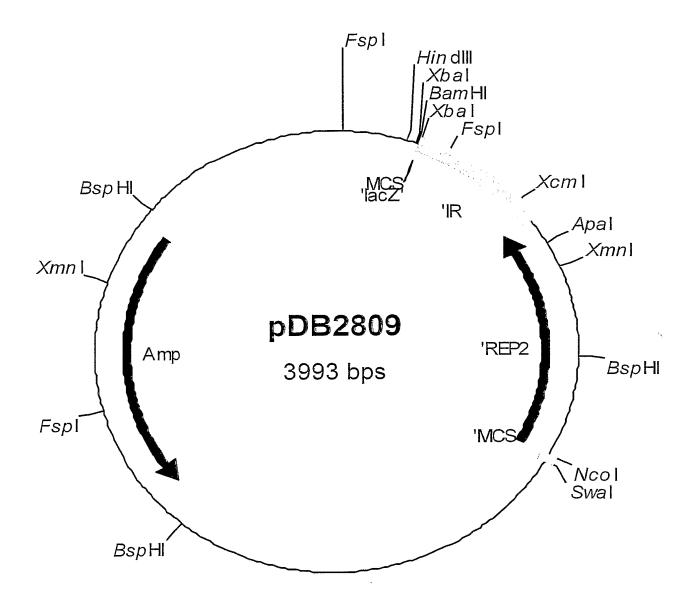


Figure 40

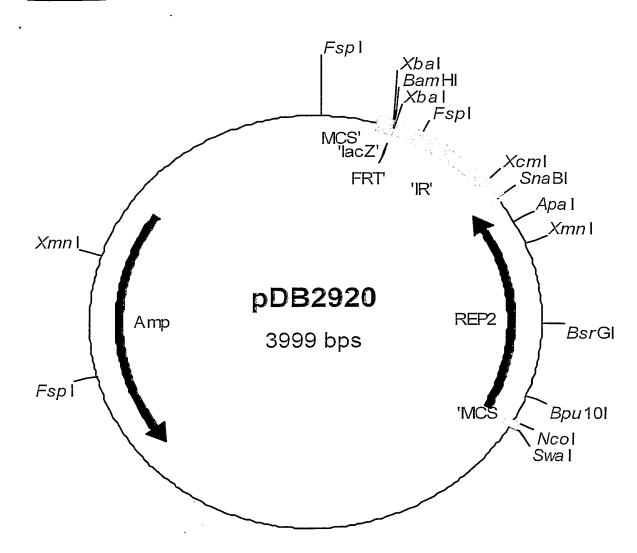


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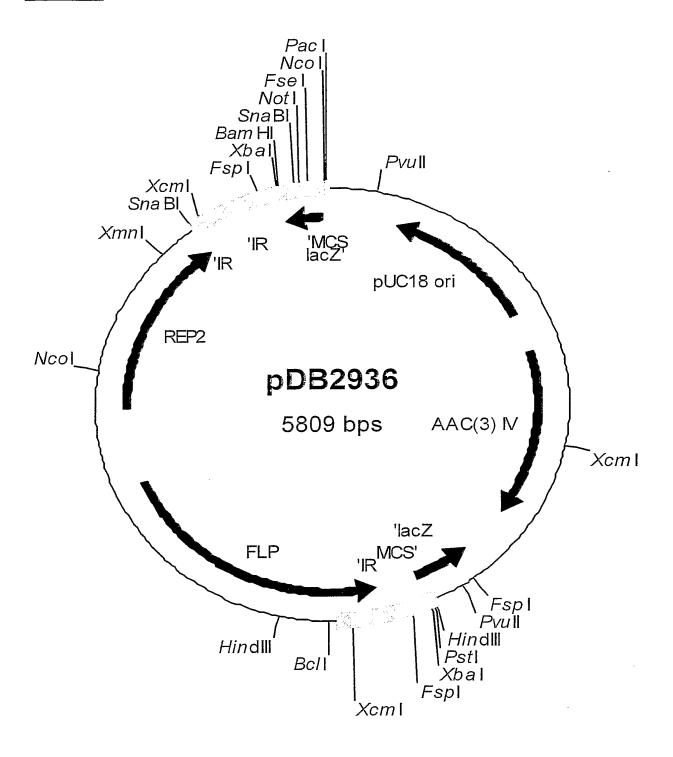


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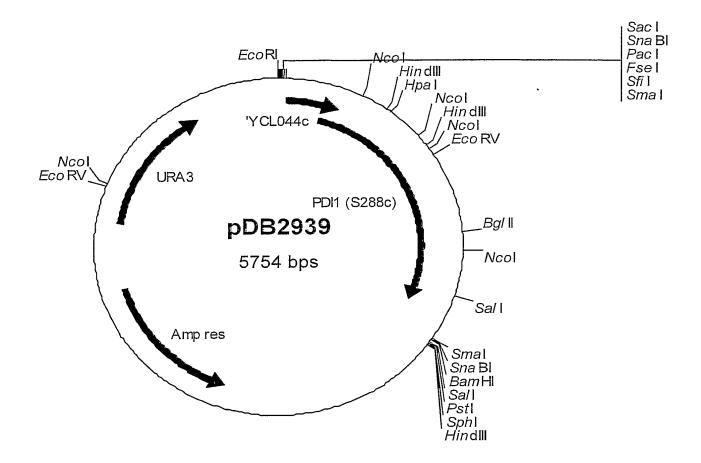


Figure 43

PCR Primers DS248 and DS250 for amplification of S. cerevisiae PDII genes

SmaI Sfil FseI PacI SnaBI SacI ECORI DS248

5'-GICAGAAIIC GAGCICIACG IATTAAITAA GGCCGGCCAG GCCCGGGCIA GICTCITITI CCAAITTGCC ACCGIGIAGC AITTTGTIGT-3' >>......'YCL044c'....

DS250

SnaBI ---+--BamHI SmaI -+---- 5'-GICAGGAICC TACGTACCCG GGTAAGGCGT ICGTGCAGTG IGACGAATAT AGCG-3'

Figure 44

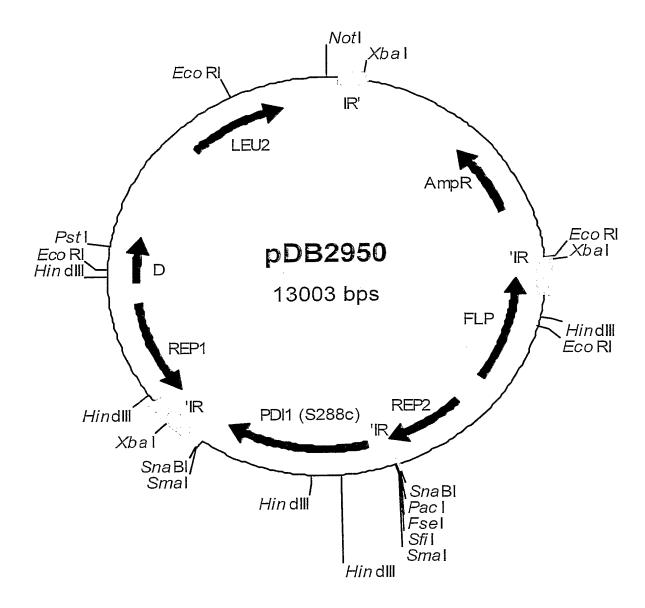


Figure 45

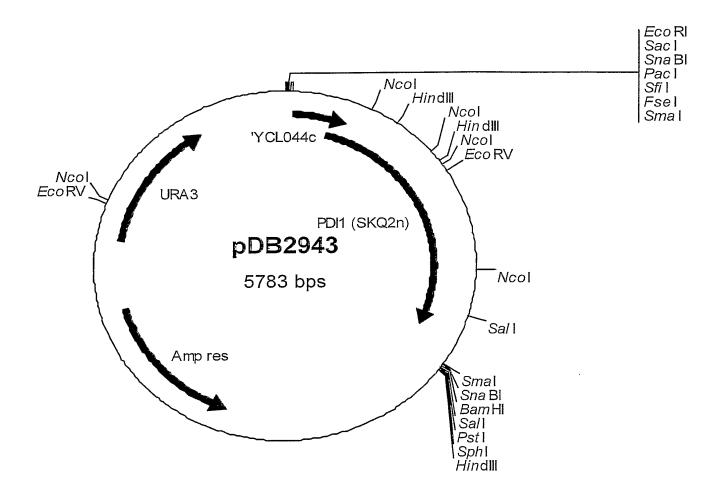


Figure 46

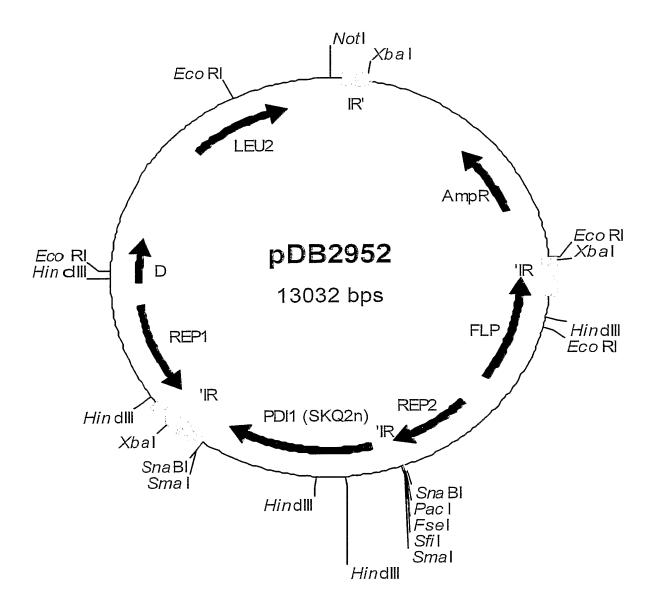
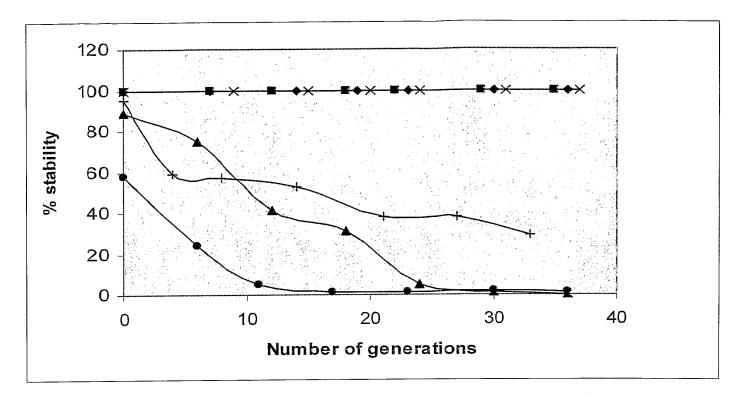
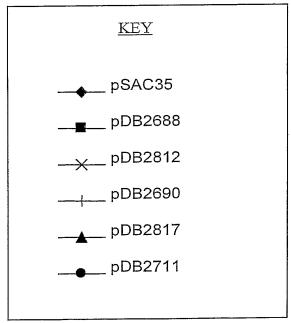
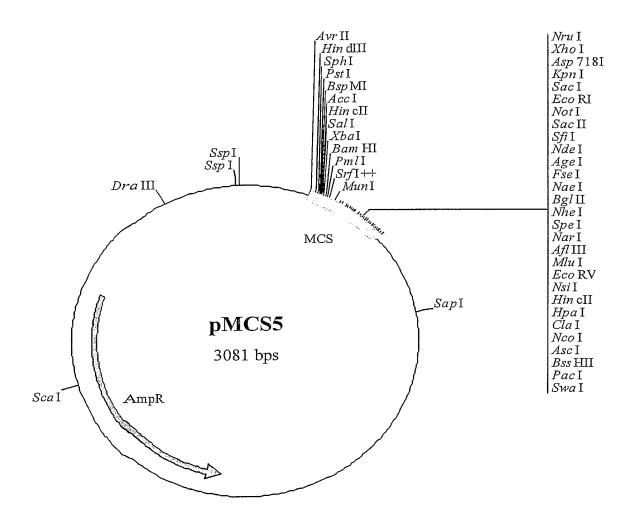
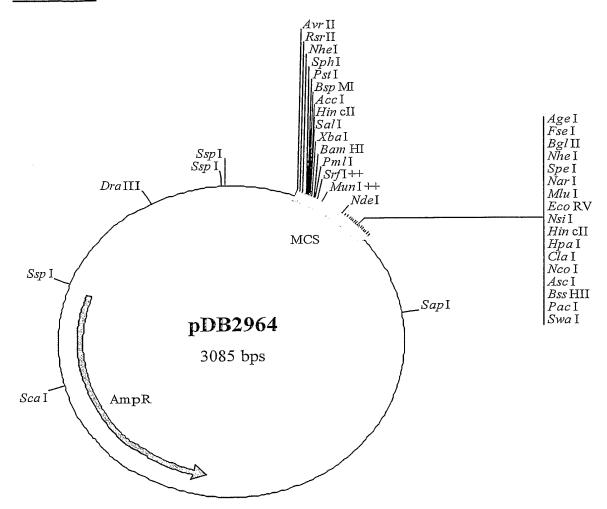


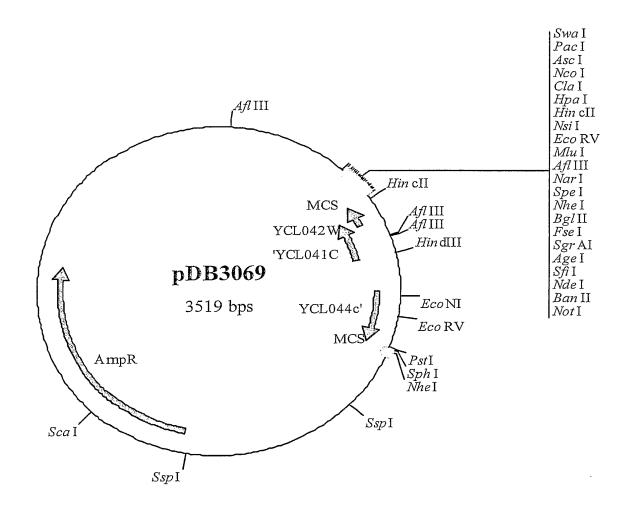
Figure 47

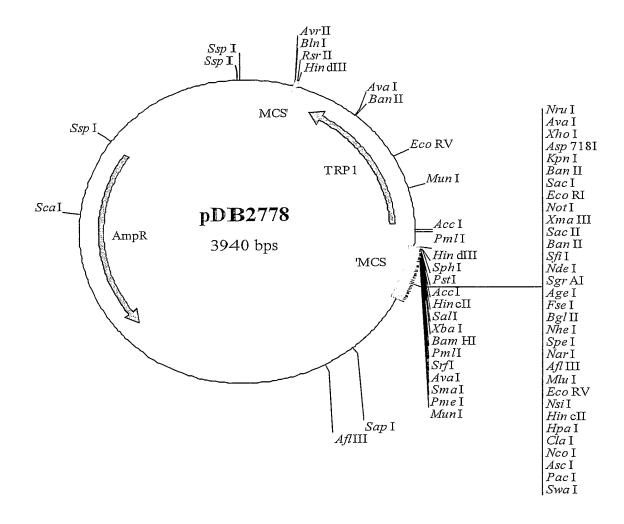


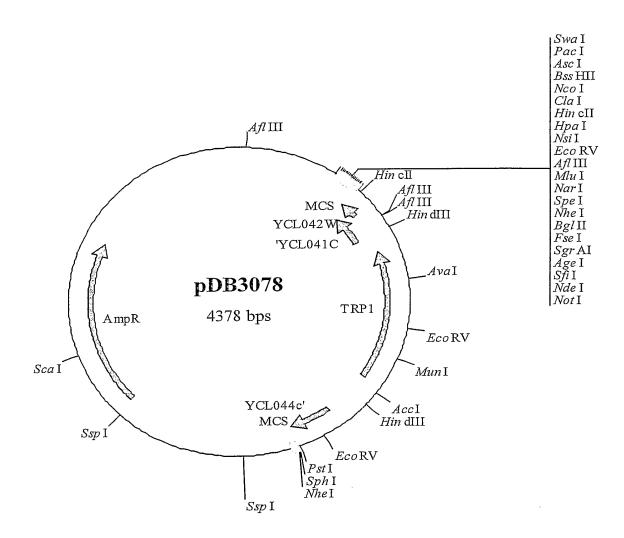


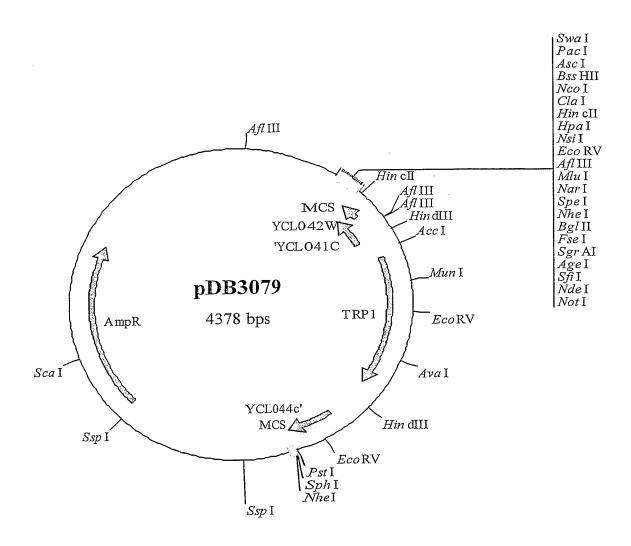


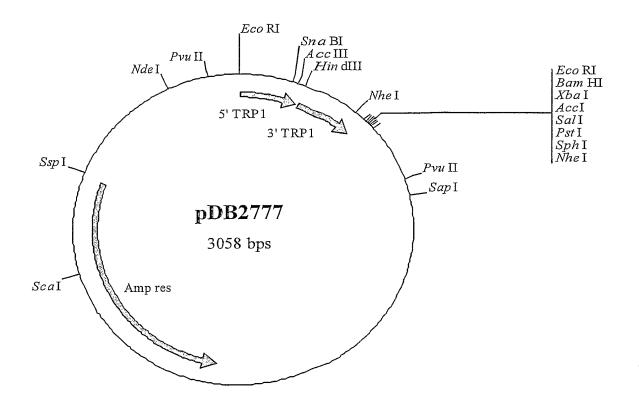












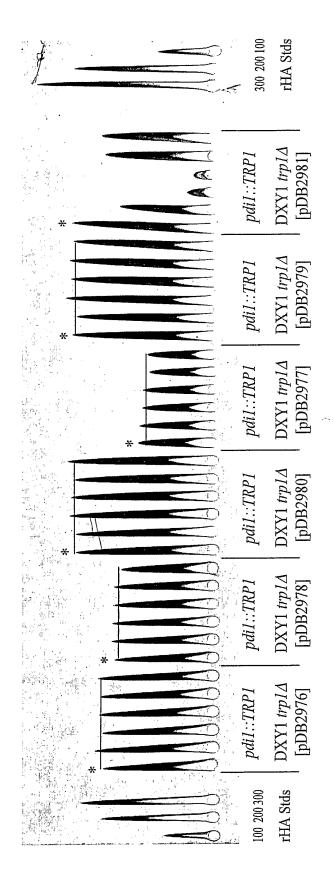


FIGURE 55

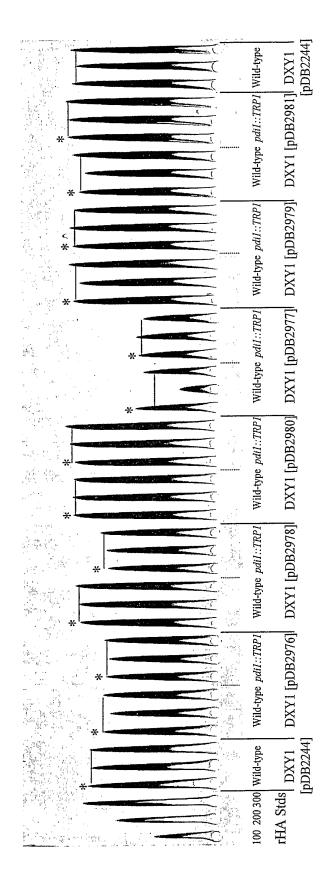


FIGURE 56

Figure 57

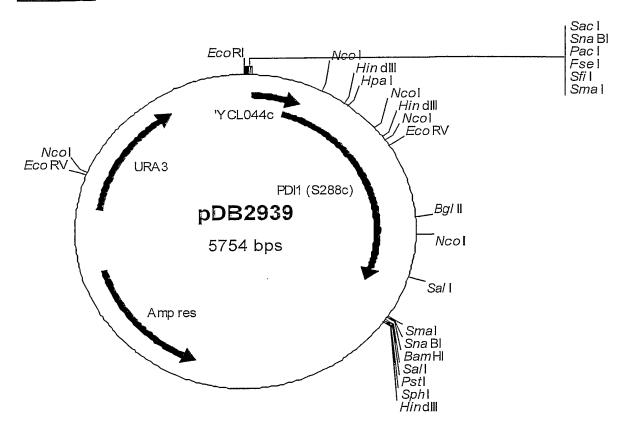


Figure 58

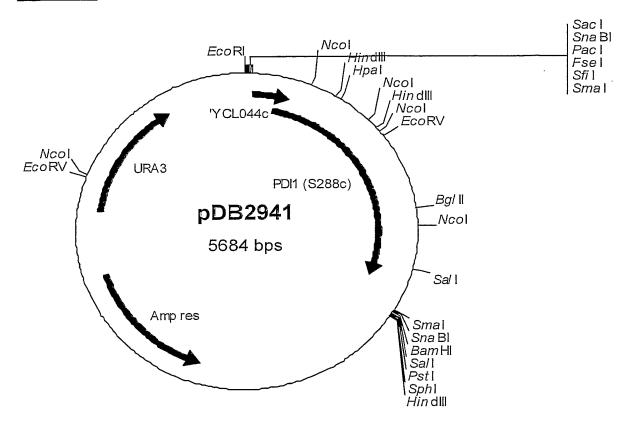
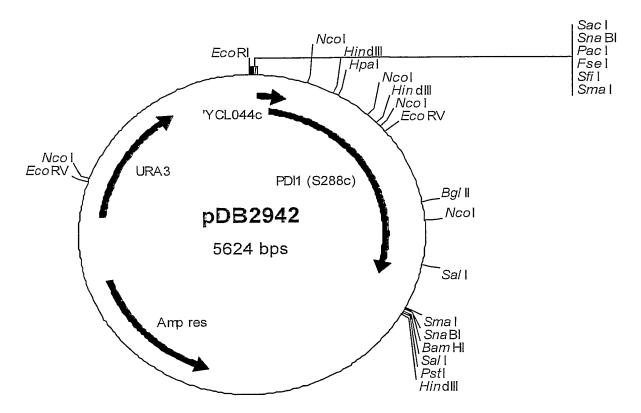
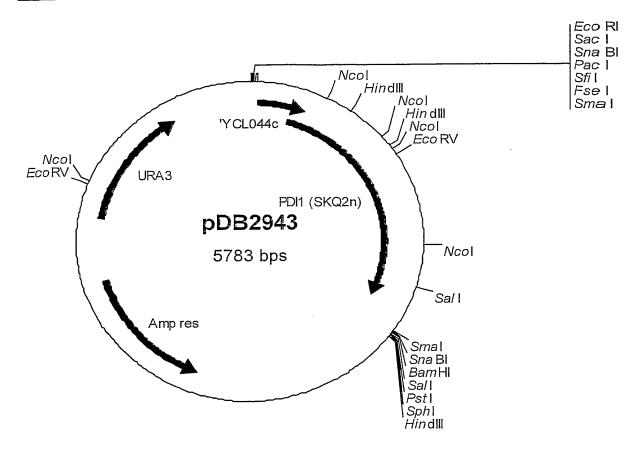


Figure 59



## Figure 60



## Figure 61

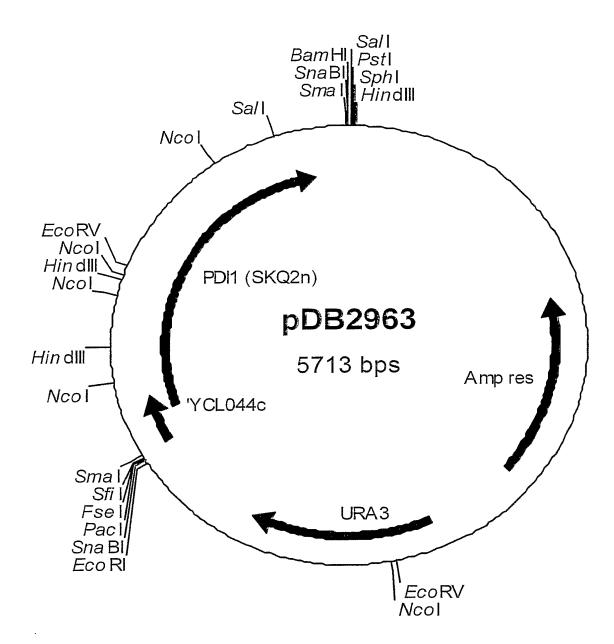


Figure 62

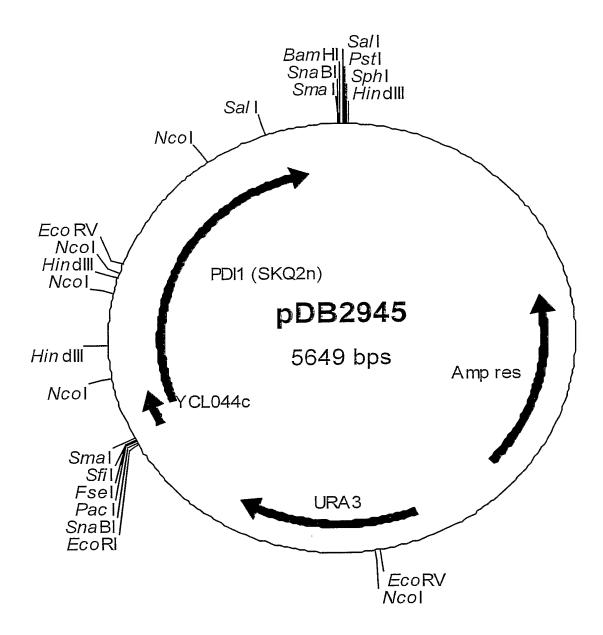


Figure 63

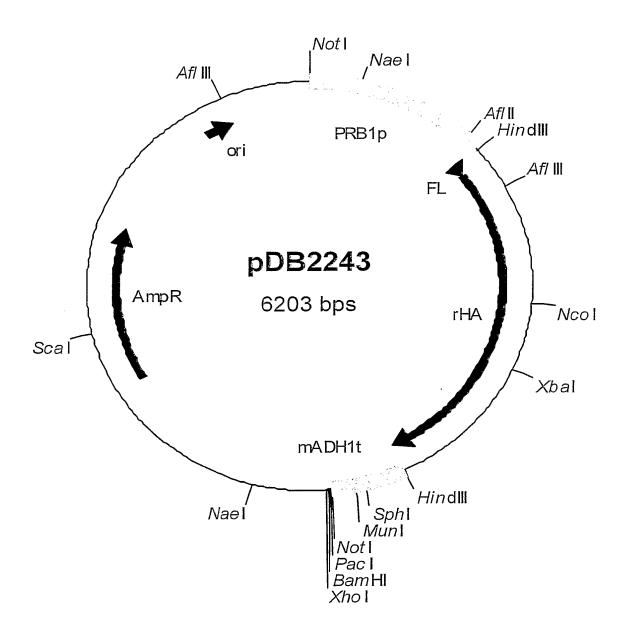
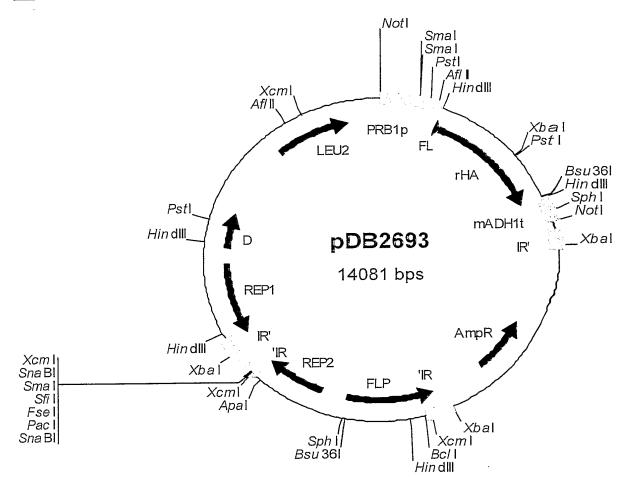
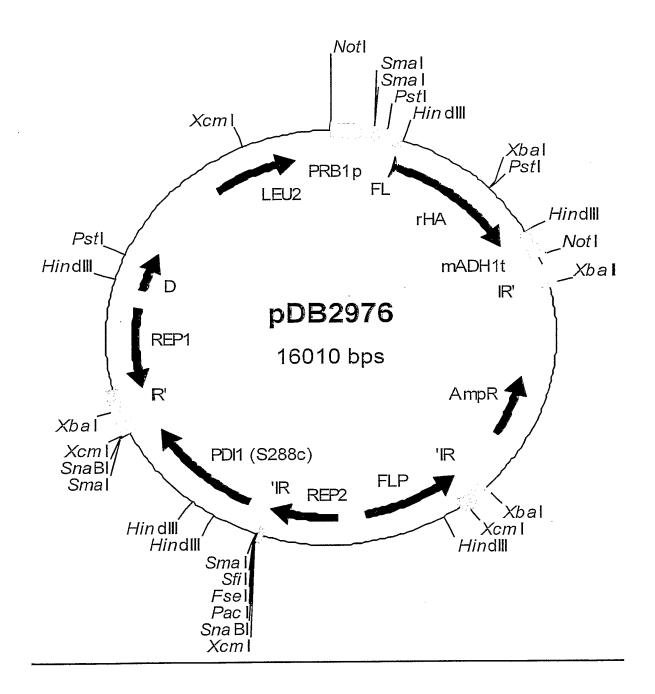
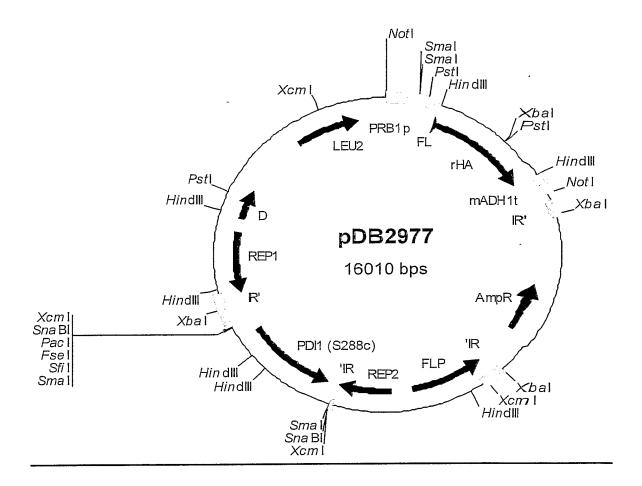
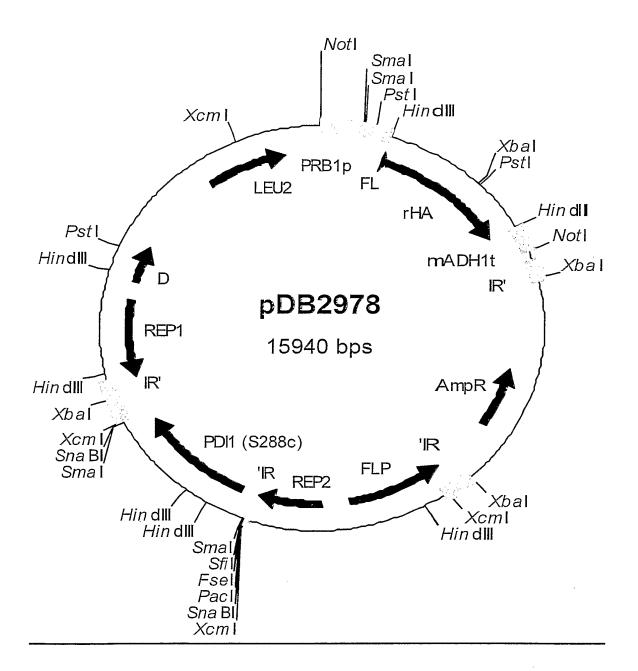


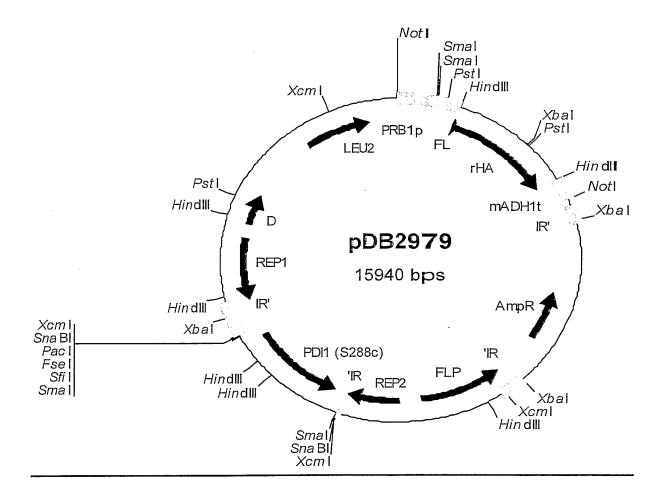
Figure 64











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